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Research Article

HEPATOPROTECTIVE ACTIVITY OF *TAMARA BHASMA* PREPARED BY TWO DIFFERENT METHODS

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ABSTRACT

Bhasmas are traditional Indian medicinal preparations in which metals/minerals undergo thermal treatment process to form oxide or sulphide form. *Tamra bhasma* is one of the potent *Rasadravya* possesses qualities like *lekhana* (scraping), *ropana* (healing), *deepana* (digestive), and indicated in diseases like, *pandu* (Anemia), *krimi* (worm), *sthoulya* (Obesity), *shotha* (inflammation), *shoola* (Abdomen pain), *yakrit-pleeha roga*,(liver-spleen disorders) etc. In *rasagranthas* different methods are mentioned for the preparation of *tamra bhsma*. Two different methods were selected for present study. Keeping its immense qualities in view hepatoprotective effect of *tamra bhasma* against carbon tetrachloride (CCL_4) induced toxicity was studied in albino rats. Alteration in the level of biochemical markers of hepatic damage like SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase), Alkaline phosphates, total serum, and Billirubin were tested.

Keywords: *Tamra Bhasma, Somanathi Tamra Bhasma*, Biochemical Parameters, Histopathology

INTRODUCTION

Ayurvedic drugs play a major role in the treatment of hepatic disorders. In the absence of reliable liver protective drugs in contemporary sciences, numbers of ayurvedic formulations are used to treat hepatic disorders in traditional system of medicine. For present study *tamra bhasma* was selected, as it is indicated in *yakrit-pleeha roga*[1] (liver-spleen disorders). To prepare tamra bhasma many methods were explained in *Rasagranthas. Somanathiya tamra bhasma*[2] also one among them which is mentioned by *Acharya Somanatha* and indicated in *shoola* (Pain in abdomen), *panduroga (Anemia), jwara (fever), pleeha-yakrit roga* (liver-spleen disorders)., *agnimandya* (indigestion), *prameha (Diabetes)* etc[2]. So present study intended to evaluate comparative hepatoprotective activity of tamra bhasma and *somanathiya tamra bhasma*. CCl_4 was used to induce hepatotoxicity in rats. For evaluation parameters selected were biochemical markers of hepatic damage like SGPT, SGOT, Alkaline phosphates, total serum, and Billirubin and Histopathological study also carried out.

MATERIALS AND METHODS

***Tamra bhasma*[3]:**

Shodhita Tamra (Purified copper) – 200g *Kajjali* (Mercury+Sulphur) – 400g

Procedure: *Kajjali* was mixed with nimbu rasa applied to the *Tamra patra* (thin copper sheet) and dried well. Then *kajjali lipta Tamra patras* were kept in *sharava* (casserole), and closed with another *sharava. Sandhibandhana* (sealing) was done with mud smeared cloth. Thus prepared *Samputita Sharava* (sealed casserole) was subjected to *Gajaputa* (heating system). After *Swangashita* (self cool) material was removed out of *sharava* and it was powdered in *khalwayantra* (mortar & pestle). Then again *kajjali* and *nimbu rasa (Lemon juice)* were mixed triturated well prepare *chakrika (pellets)*. Dried *chakrikas* were kept in *sharava samputa* subjected to *gajaputa*. Like this total seven *putas* were required to prepare *bhasma*. Thus prepared *bhasma* was subjected to *bhasma pareeksha* and physico-chemical analysis. 250 g loss was observed.

Preparation of *Somanathiya Tamra Bhasma*[4]:

1) *Shodhita Tamra* – 200g
2) *Kajjali* made of *Parada (mercury), Gandhaka (sulphur), Haratala* (As_2S_3) *& Manahshila* (As_2S_2) – 550g

Procedure: A *Sharava* was taken and some amount of *Kajjali* was sprinkled. Above this *Tamra patra* was spread. Over *Tamra patra* again *Kajjali* was spread alternatively. Thus prepared *sharava* was closed with another *sharava* and sealed with mud smeared cloth. This *Samputita Sharava* was subjected to *Gajaputa*. After *Swangashita* material was removed out of *sharava* and it was powdered in *khalwayantra* (mortar & pestle).. Then *nimbu rasa* (lemon juice) was mixed triturated well prepare *chakrika.* Dried *chakrikas* were kept in *sharava samputa* (sealed casserole) subjected to *gajaputa*. Like this total three *putas* (heating system) were required to prepare *bhasma*. Thus prepared *bhasma* was subjected to *bhasma pareeksha* (test of perfectness) and physico-chemical analysis. 400 g loss was observed.

Table 1: *Bhasma Pareeksha*[5,6,9,10]:

Sl. No	TEST	OBSERVATION	
		TAMRA BHASMA	**SOMANATHI TB**
01	*Varna* (Colour)	Black	Black
02	*Gatarasatvam* (Tastelessness)	Non- Perceivable	Non- Perceivable
03	*Sparsha* (Touch)	*Mrudutva*(soft) was felt by simple touch with finger tips	*Mrudutva* (soft) was felt by simple touch with finger tips
04	*Gandha* (Smell)	Non- Perceivable	Non- Perceivable
05	*Rekhapurnatva* (fills the furrows of finger)	The *Bhasma* was rubbed in between first finger and thumb. It penetrates into the furrows of the fingers - Positive	The *Bhasma* was rubbed in between first finger and thumb. It penetrates into the furrows of the fingers – Positive
06	*Varitaratva* (floating property on water surface)	A small amount of *Bhasma* was carefully sprinkled in beaker full of water. It was found that total portion of *Bhasma* was floating on the water surface - Positive	A small amount of *Bhasma* was carefully sprinkled in beaker full of water. It was found that total portion of *Bhasma* was floating on the water surface – Positive
07	*Nischandratvam* (absence of metallic lusture)	The *Bhasma* observed in bright sunlight. It was not having any lusture – Positive	The *Bhasma* observed in bright sunlight. It was not having any lusture - Positive
08	*Amlapareeksha* (sour test)	For *Bhasma* when putted some drops of Lemon juice it does not change to green.	For *Bhasma* when putted some drops of Lemon juice it does not change to green.

Animals: Albino rats were used as experimental model. Albino rats of either sex weighing between 150-200g breeds in animal house were selected. They were housed individually in polypropylene cages in well-ventilated rooms. The rats were kept under observation for seven days with standard laboratory diet. After which they were examined for their normal health and then subjected to experimental study.

24 animals were selected, which have been divided into 4 groups. Each group with six animals was kept in separate cages after proper labeling for identity.

Hepatoprotective activity: Animals were divided into four groups. Each group consists of six animals. Both the trial drugs were given in the form of aqueous suspension. 225 g of *Bhasma* was added to 40 ml of 2% twine 20 (suspending agent) solution and mixed well. Each 0.2 ml contains 1.125 mg of *Bhasma*. Carbon Tetrachloride (CCl_4) was given at the dose of 0.7ml/kg, intra peritoneal (i.p) for first five days to induce liver damage[7].

Table 2: Showing Experimental Protocol

Group	Pre-treatment dose/route	Duration in days	Days of withdrawal of blood	Purpose
G 1	Vehicle	1-5	6^{th}	Control
G 2	CCl_4 0.7ml/kg i.p	1-5	6^{th}	Liver damage
G 3	CCl_4 0.7ml/kg i.p	1-5	11^{th}	Curative
	Tamra Bhasma 1.125 mg (aqueous suspension) orally	6-10		
G 4	CCl_4 0.7 ml/kg i.p	1-5	11^{th}	Curative
	Somanathiya Tamra Bhasma 1.125 mg (aqueous suspension) orally	6-10		

Biochemical parameters[8]: Blood samples were withdrawn from albino rats at different intervals that are on 6^{th} day for 1^{st} and 2^{nd} group while on 11^{th} day for the remaining two groups (3^{rd} and 4^{th}). The serum enzyme activity was estimated by standard bio-chemical procedure using an auto-analyzer for all the groups.

Following enzyme levels were estimated for the study.

1. Alkaline phosphatase.
2. SGOT (Serum glutamate oxalacetate transaminase)/AST

3. SGPT (Serum glutamate pyruvate transaminase)/ALT
4. Total serum bilirubin.
5. Serum albumin.

Histopathological study: Animals were sacrificed on the day of withdrawal of blood from all the four groups and liver was isolated, sliced and washed with saline. Then it was preserved in 10% of formalin, for histopathological studies. Later the microscopic slides of the liver cells were photographed. Routine staining procedures using haematoxylin and eosin stain were done in the histopathological studies.

STATISTICAL ANALYSIS:

The analysis of experimental data, using anova for significance of the difference between averages of the different groups reveals.

Comparing G2 (Liver damage) and G3 (Treated with TB) there is a significant different in their effect at levels indicated in the table except SGOT and ALP more over in G3 values are reduced.

Comparing G2 (Liver damage) and G4 (Treated with STB) there is a significant different in their effect at levels indicated in the table for all tests.

Comparing G3 (Treated with TB) and G4 (Treated with STB) there is significant different in their effect at levels indicated in the table except SGOT and ALP.

The above analysis showed that both TB (*Tamra Bhasma*) and STB (*Somanathi Tamra Bhasma*) are effective in treatment. Among the two STB is more effective in treatment.

RESULTS

Table 3: Showing summary of Biochemical values of all groups

Group	No of Animals	Drug and Dose	Duration of Treatment in days	Bio-chemical Parameters (mean & ± SEM)				
				SGPT	SGOT	ALP	T-Bil	Albumin
G1 Control	6	Vehicle	1-5	59.30 ±2.410	432.90 ±26.73	138.90 ±82.40	0.791 ±0.011	3.50 ±0.057
G II CCL4	6	CCl_4 0.5ml/kg	1-5	287.89 ±29.700	764.27 ±76.93	478.69 ±11.06	0.98 ±0.004	2.30 ±0.036
G III Treated with TB	6	CCl_4 0.5ml/kg	1-5	198.60 ±9.430	678.40 ±8.63	328.90 ±6.04	0.95 ±0.003	3.36 ±0.055
		TB 1.125 mg	6-10					
G IV Treated with STB	6	CCl_4 0.5ml/kg	1-5	97.89 ±6.290	497.39 ±11.06	159.00 ±9.91	0.84 ±0.004	2.63 ±0.042
		STB 1.125mg	6-10					

Table 4: Showing the comparison of effect of toxic group with treated groups (By means of t values)

Parameters	G2 vs G3	G2 vs G4	G3 vs G4
SGPT	5.60 **	11.919 ***	6.31 **
SGOT	2.078	6.459 ***	4.381 *
ALP	3.569	7.616 ***	4.648 *
T- Bil	4.699 *	19.785 ***	15.08 ***
Alb	21.82 ***	6.82 ***	15.00 ***

* - Medium significant table value (P< 0.05)
** - Medium significant table value (P< 0.01)
*** - Highly significant table value (P<0.001)

Histopathology Report:

Group G1: Liver sections of normal control rats showing: normal hepatic cells with well preserved cytoplasm; well brought out central vein; prominent Nucleus and nucleolus.

Group G2: Liver section showing: massive fatty changes, necrosis, ballooning degeneration, and broad infiltration of the lymphocytes and kupffer cells around the central vein and the loss of cellular boundaries.

Group G3: Photomicrograph of liver section, showing central vein surrounded by hepatocytes with sinusoidal dilation with occasional inflammatory cells. No hepatic necrosis was seen around central vein or in the Central zone.

Group 4: Liver section, showing: well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus.

DISCUSSION

In text for *Tamra bhasma* 3 putas & for *Somanathiya Tamra bhasma* 1 puta is mentioned. But practically it requires 7 & 3 respectively. *Somanathiya tamra bhasma* requires less number of *puta* because it contains *haratala, manasila* as a *maraka dravya*. pH report showed that it was 7.33 in *Tamra bhasma* whereas in *Somanatiya Tamra bhasma* it was 5.09.

In group 2 all the biochemical values SGPT, SGOT, ALP, T-Bil, Albumin were highly increased except the serum albumin which is decreased. The histopathology study of Liver of this group showed severe degree necrosis, ballooning degeneration, and broad infiltration of the lymphocytes and kupffer cells around the central vein and the loss of cellular boundaries. It indicates that in course of CCl_4 administration leads to functional defects of the hepatocytes & multiple biochemical took place.

Biochemical & histopathological observations shown that these two trial drugs significantly efficient in protecting the liver. Except TB in case of SGOT and ALP. To reveal the curative effect of treated groups (G3 & G4) The Bio chemical values and Histopathological study of Liver were analyzed by comparing between the groups like G2 with G3 and G2 with G4. G2 with G3 shows significant difference except in case of SGPT & ALP. (i.e $P > 0.05$). G2 with G4 shows significant difference with all Biochemical values. ($P< 0.001$) It indicates that these two Trial drugs have significant effect in protecting liver.

The Histopathology of G3 shows central vein surrounded by hepatocytes with sinusoidal dilatation with occasional inflammatory cells. No hepatic necrosis was seen around central vein or in the Central zone. Group 4 shows well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus.

Among the two when we analyzed we come to know that the effect is not equal and biochemical observations reveal that the values of G4 are closer to the Normal (G1) than G3. So *Somanathiya Tamra bhasma* is more effective than *Tamra bhasma.*

CONCLUSION

By comparing Biochemical, Histological and Statistical analysis both *Tamra bhasma* and *Somanathiya Tamra bhasma* have significant therapeutic effect on hepatotoxicity. *Somanathiya Tamra bhasma* is highly effective among both the *bhasmas*.

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